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(54) Title: ACTIVE OXYGEN ANNIHILATING AGENT

(57) Abstract

Disclosed is a novel active oxygen annihilating agent in the form of a syrup or powder suitable as an additive to health foods to reduce noxious influences of active oxygen against human body. The active oxygen annihilating agent is prepared from locust bean pods having heretofore little utilizability but to be discarded as an agricultural waste material. Thus, crushed particulates of locust bean pods are subjected to an extraction treatment with water or ethyl alcohol and the extract solution is concentrated by evaporating at least a part of the solvent to give a syrup which can be freeze–dried or spray–dried into a powder. The syrup can be processed, for example, into a delicious jam for spreading over bread.

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DESCRIPTION

ACTIVE OXYGEN ANNIHILATING AGENT

Technical Field

The present invention relates to an active oxygen annihilating agent as a foodstuff additive and a method for the preparation thereof by utilizing an agricultural waste material as well as to a foodstuff containing the active oxygen annihilating agent.

Background Art

"Active oxygen" is a conceptual term which generally means several oxidative species including superoxide anions, oxygen radicals, hydroxyl radicals, hydrogen peroxide and the like having, when taken up from outside into or generated inside of a human body, adverse influences on the health condition of the human body such as peroxidation of lipids and mutation of DNAs to give increasing damages on living cells slowly but steadily disturbing normal activities to maintain the living body and finally leading to formation of malignant tumors and senescence acceleration. Accordingly, it is an important problem to reduce take-up amounts of active oxygen into human body.

Advantageously, living bodies in general have a mechanism to render the active oxygen innoxious which suppresses appearance of the above mentioned unsound or diseased condition of the living body. This suppressing activity against the adverse effects of active oxygen is largely by virtue of the superoxide dismutase, referred to as SOD hereinafter, as a kind of enzymes, of which abundance level in the living body widely differs among individuals depending on the ages, races, living environments and other factors. When the inherent SOD level in a person is so low as to involve a health problem, the overly active oxygen can

be annihilated by factitiously supplying a substance which exhibits the same activity as the SOD from outside at least temporarily. Such a substance is usually referred to as an SOD-like substance.

Various foods and beverages are known to contain SOD-like substances including polyphenol compounds in red wine and cacao beans and catechins in green tea. The content of the SOD-like substance in these foodstuffs, however, is so low that they are practically inapplicable to an acute symptom due to excess of active oxygen requiring an unduly large amount of the foodstuff intake. The SOD-like substances in foodstuffs cannot be concentrated without adversely affecting the tastiness or flavoriness necessarily to degrade the quality of the foodstuff.

Accordingly, the inventors have conducted extensive screening tests of a variety of natural products with an object to uncover a material exhibiting an SOD-like activity to direct their attention to locust bean pods.

Locust bean is a leguminous plant having a scientific name of Ceratonia siliqua L. and growing in the Mediterranean coastal districts such as Italy, Spain and Portugal. The major application of locust beans is as a food additive including a powder obtained by pulverizing the seed albumen of locust beans to serve as a thickening and stabilizing agent called a locust bean gum.

Needless to say, production of locust beans is necessarily accompanied by a corresponding amount of locust been pods as an agricultural waste material. Despite the so large amount of locust bean pods as a by-product of locust beans, only a very small portion of the annual production of locust bean pods is utilized, for example in the United States, as a cocoa substitute or a coating material on confectionery and almost all of the locust bean pod production is merely discarded or, at best, utilized as a livestock feed. Accordingly, value-added utilization of locust bean pods is an important issue heretofore in the agricultural industry. Thus, the inventors have directed their attention

to locust bean pods as one of the raw materials in their screening work to uncover an efficient and inexpensive source material of an SOD-like substance.

Disclosure of Invention

The present invention accordingly provides a novel active oxygen annihilating agent having SOD-like activity which is an extract from locust bean pods in the form of a syrup or powder. The invention also provides a foodstuff admixed with the active oxygen annihilating agent.

The above mentioned active oxygen annihilating agent having SOD-like activity is prepared from locust bean pods by a method which comprises the steps of:

(a) crushing locust bean pods into particulates;

(b) bringing the particulates of locust bean pods into contact with a solvent as an extractant to form an extract solution of soluble matter in the locust bean pods;

(c) separating solid remnant from the extract solution; and

(d) removing at least a pat of the solvent from the extract solution into a consistency of a syrup or into a powder.

The active oxygen annihilating agent in the form of a syrup or powder prepared in the above described manner contains, in addition to the active oxygen annihilating ingredient, a good amount of saccharides so that the syrup or powder can be added to a foodstuff of any types as a sweetening material besides the effect of active oxygen annihilation.

Best Mode for Carrying Out the Invention

The inventors conducted, as an outset, a chemical analysis of locust bean pods to determine various saccharide compounds including monosaccharides, disaccharides and sugar alcohols. The results were as follows, where the saccharide contents per 100 g of dried locust bean pods are given in five ratings with the respective symbols of: (*) for less

than 0.05 g; (**) for less than 0.3 g; (+) for 0.05 to 1 g; (++) for 1 to 10 g; and (+++) for more than 10 g.

Monosaccharides: fructose (++); glucose (++); arabinose (+); xylose (+); mannose (+); and galactose (*)

Disaccharides: sucrose (+++); maltose (**); and lactose (*)

Sugar alcohols: xylitol (**); mannitol (*); sorbitol (+); and maltitol (*)

Namely, the saccharide constituent of locust bean pods consists mostly of sucrose, fructose and glucose so that the extract from locust bean pods has little problem on the health of human bodies even when taken in a relatively large amount. It is a quite unexpected discovery that, while absolutely no SOD-like activity can be noted in the germs and seed albumens of locust beans per se, very strong active oxygen annihilating activity is exhibited by the extract from locust bean pods.

In the preparation of the inventive active oxygen annihilating agent, locust bean pods are subjected to solvent extraction by using an extractant solvent to give an extract solution. Although the extract solution as such exhibits a certain level of active oxygen annihilating activity, it is advantageous that at least a part of the solvent is removed from the extract solution to give a syrupy liquid containing the active substance in an increased concentration. Further, the syrupy liquid can be freeze-dried or spray-dried to give an active oxygen annihilating agent in the form of a powder.

In the first step of the inventive method, locust bean pods are dried and crushed into particulates of a 0.01 to 1.5 mm or, preferably, smaller than 1.0 mm particle diameter. When the particulates are too coarse, a decrease is resulted in the efficiency of extraction of the active substances in the step of solvent extraction.

The particulates of locust bean pods are then brought into contact with an extractant solvent to effect extraction of the active substances to give an extract solution. Water is preferable as the extractant solvent although some organic solvents can be used for the purpose including alcoholic solvents such as methyl alcohol, ethyl alcohol, n-propyl alcohol, isopropyl alcohol, n-butyl alcohol, isobutyl alcohol, sec-butyl alcohol and tert-butyl alcohol, acetone, chloroform and n-hexane. These solvents can be used either singly or as a mixture of two kinds or more, if miscible. If necessary to shorten the extraction time, it is optional to conduct the extraction treatment at an elevated temperature.

When hot water is used as the extractant solvent for the extraction treatment of the particulates of locust bean pods, the amount of water is in the range from 5 to 50 times by weight of the particulate pods. The thus formed extraction mixture is kept under agitation at a temperature of 80 to 100 ℃ or, preferably, at about 100 ℃ for 1 to 60 minutes or, in most cases, for 5 to 20 minutes followed by filtration of the mixture to give a clear extract solution by removing the solid remnant. When the extraction treatment is undertaken at room temperature, the extraction mixture is agitated for 3 to 24 hours followed by filtration.

The extract solution obtained in the above described manner is then subjected to a concentrating treatment by evaporating at least a part of water as the solvent at a temperature of 60 to 100 ℃ or, preferably, at about 70 ℃ or under a reduced pressure to give a syrupy liquid which exhibits strong active oxygen annihilating activity. This syrupy liquid can be further subjected to a freeze-drying treatment or spray-drying treatment into a powdery form by completely removing the solvent.

Use of an alcoholic solvent has an advantage as compared with water that extraction of the saccharide materials from the locust bean pods can be decreased relative

to the SOD-like active substances so that the active oxygen annihilating agent contains the SOD-like substances in an increased concentration relative to the saccharides. It is of course possible to control relative extraction of the saccharides and the SOD-like active substances by modifying the polarity of the extractant solvent with addition of water to an alcoholic solvent depending on the active oxygen annihilating agent as desired.

When ethyl alcohol is used as at least a part of the extractant solvent, particulates of locust bean pods are added to 5 to 20 times by weight of an extractant solvent, which may be absolute alcohol or a mixture of water and ethyl alcohol containing 60% by weight or less or, preferably, 30% by weight or less of water, and the extraction mixture is agitated at room temperature for 15 minutes to 48 hours or, preferably for at least 24 hours followed by filtration to give a clear extract solution by removing the solid remnant. The extract solution is subjected to a concentrating treatment by evaporating ethyl alcohol under reduced pressure to give a syrupy liquid which can be freeze-dried or spray-dried into a powder.

When an organic solvent other than ethyl alcohol is used as the extractant solvent, it is necessary that the inventive active oxygen annihilating agent is prepared in the form of a dry powder by completely removing the solvent in the concentrating treatment in consideration of the physiological effects of the solvent on the human body.

The preparation form of the inventive active oxygen annihilating agent, which can be a syrupy liquid or a powder, naturally depends on the particularly intended application. A syrupy preparation can be diluted as such with cold or hot water or with an alcoholic drink to give a health beverage. A powder preparation can be sprinkled as such over a food or can be used as an additive ingredient in food processing.

In the following, the present invention is described in more detail by way of Examples.

Example 1.

A 50 g portion of particulates of dried locust bean pods was added to 250 g of hot water to give an extraction mixture which was boiled for 15 minutes and then filtered by using filter paper to give a filtrate. This aqueous solution was subjected to the test of active oxygen annihilating activity by the method of electron spin resonance (ESR) spectrometry to find an activity of 1.7×10^3 U/g.

The aqueous solution was concentrated by heating to evaporate water into the form of a thick syrup of pasty consistency having a saccharide content of about 80%, which was again subjected to the ESR measurement to obtain a very high activity value of 1.0×10^4 U/g.

This syrup had a good taste with sweetness and flavor resembling black raw sugar and was delicious for eating in a manner like a conventional jam spread over bread or in place of a maple syrup on pan-cakes.

A tasty drink with a flavor like cocoa could be obtained by dissolving the above prepared syrup in an appropriate volume of hot water under agitation.

Example 2.

The extract syrup of locust bean pods prepared in Example 1 was diluted with hot water to make a diluted syrup having a final saccharide content of 50% by weight, from which a pasty sweet having a jam-like consistency was prepared in a formulation including 45% by weight of the syrup calculated as the saccharide content, 1% by weight of xanthan gum and 0.1% by weight of docosahexaenoic acid (DHA), the balance being water.

This pasty sweet had a consistency suitable for spreading over bread and had a masking effect on the offensive taste inherent in DHA. Accordingly, the pasty sweet could be evaluated as a valuable health food having active oxygen annihilating activity derived from locust bean pods without distastefulness due to DHA.

Example 3.

The locust bean pods extract in the form of a syrup of about 80% saccharide concentration prepared in the same manner as in Example 1 was diluted with hot water to give a diluted extract solution of 10% saccharide concentration. A 1000 g portion of the diluted extract solution was taken in a dish tray and frozen at -85 ℃ and subjected to freeze-drying for 24 hours in a vacuum freeze-dry apparatus to find that the syrup was completely dried into the form of a cake which could easily be disintegrated into a light and fluffy powder of very fine particles.

A 100 g portion of an unsweetened yogurt was admixed with 3 g of the thus freeze-dried powder to find that the powder could readily be dissolved in the yogurt and had moderate sweetness. Thus, the extract from locust bean pods serves not only as an active oxygen annihilating agent but also as a sweetening agent.

Industrial Applicability

An active oxygen annihilating agent suitable as an additive in health food products can be produced from locust bean pods which hitherto had almost no way of utilization but to be discarded as an agricultural waste material.

CLAIMS

1. An active oxygen annihilating agent having an activity of superoxide dismutase which is an extract from locust bean pods in the form of a syrup or powder.

2. A method for the preparation of an active oxygen annihilating agent in the form of a syrup or powder which comprises the steps of:
(a) crushing locust bean pods into particulates;
(b) bringing the particulates of locust bean pods into contact with a solvent as an extractant to give an extract solution of soluble matter in the locust bean pods;
(c) separating solid remnant from the extract solution; and
(d) removing at least a part of the solvent from the extract solution.

3. The method for the preparation of an active oxygen annihilating agent in the form of a syrup or powder according to Claim 2 in which the solvent as the extractant is water.

4. The method for the preparation of an active oxygen annihilating agent in the form of a syrup or powder according to Claim 3 in which the amount of water as the extractant solvent is in the range from 5 to 50 times by weight of the locust bean pods.

5. The method for the preparation of an active oxygen annihilating agent in the form of a syrup or powder according to Claim 3 in which step (b) for contacting locust bean pods with the extractant solvent is conducted at a temperature in the range from 80 to 100 ℃ for 1 to 60 minutes.

6. The method for the preparation of an active oxygen annihilating agent in the form of a syrup or powder

according to Claim 2 in which the active oxygen annihilating agent is in the form of a powder and step (d) for removing the solvent from the extract solution is conducted in two steps of (d1) removing a part of the solvent from the extract solution to give a syrupy liquid and (d2) subjecting the syrupy liquid to freeze-drying or spray-drying to completely remove the solvent.

7. The method for the preparation of an active oxygen annihilating agent in the form of a syrup or powder according to Claim 2 in which the solvent as an extractant is ethyl alcohol or a mixture of ethyl alcohol and water containing 60% by weight or less of water.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 99/01397

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/20 A23L1/211

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Section Ch, Derwent Publications Ltd., London, GB; Class D13, AN 1973-81324U XP002123527 & JP 48 043628 B (SHIMAMOTO Y AND MORI H) abstract ---	
A	DATABASE EPODOC 'Online! European Patent Office CN-A-1076727, 29 September 1993 (1993-09-29) DONGHUA INDUSTRY CORP SHENYANG: XP002123526 abstract ---	1
A	US 5 776 756 A (AKIHIKO KIMURA; ET AL) 7 July 1998 (1998-07-07) --- -/--	

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